2010 Pierce's Disease Symposium

15-17 December, San Diego

Reports from Roundtable Discussions

(Moderator, Recorder, number of participants)

A. Grapevine transformation technology (Nancy Irelan, David Gilchrist, 14 participants)

Nancy Irelan…boss
David Gilchrist….scribe
Individuals commenting: HP: Humberto Prieto; DT, David Tricoli; AD, Abhaya Dandekar, CA, Cecilia Aguero; AW, Andy Walker; GA: general agreement

This well attended discussion focused on several issues as follows:

Overall conclusions:

- **GA : methods improvement the highest priority**…Grape transformation is a huge challenge to provide materials for both basic and translational research to address current and future commercial needs . Serious investment needed!
- **GA: The overarching need is to have protocol for grape that will enable virtually any variety to be transformed with efficiency equal to that existing currently for tomato and tobacco.**

Specific points of discussion:

- Challenges offered by different commercial varieties or rootstocks. General agreement that most commercial varieties are difficult while Thompson Seedless, Freedom, Harmony and Salt Creek work ok. General agreement that cabernet will make embryonic callus be no transgenics, Chardonnay makes callus but very few (if any) transgenics. Very difficult or impossible for these two which are high priority for successful transformation…both researcher and industry.
- GA: effective and efficient methods/protocols are needed for Cabernet Sauvignon and Chardonnay. This deserves attention now. Vacuum infiltration and biolistics have not been explored in depth.
- HP said he uses advanced embryo explants-almost callus; (?Carmina) used very early embryos or a pre-embryonic tissue with success.
- CA finds it difficult to get embryos to germinate
- HP (I think) has a 4 month schedule---uses liquid flasks at 120 rpm—cylinders with baffles at a proper angle to reduce sheer stress-mass transfer minimal-then callus to agar with Agro. Then back to flask and suggested that process could work for Cabernet.

- HP described his setup in Chili. 15 people overall with 4 highly skilled people in tissue culture; 1 in charge of reactor, 2 in logisitics preparing all media, tissue culture, Agro etc,; and 1 in molecular biology.
- AW suggested priority order of rootstock transformation: 101-14, 1103B, 110R, ? St. George (not Freedom for CA, lacks phylloxera tolerance).
- AW and others: Priority order of commercial varieties: Cabernet Sauvignon, Chardonnay, Crimson, Flame seedless
- GA:First priority for development of success, high throughput, stable transformation is for the research community, then commercial and 3rd party clients
- Methodology issues; improvement, difficulties, strategies that work and don' t seem to work.
    - HP transforms Thompson Seedless from somatic embryos; noted the bioreactor he discussed in his talk; working toward dsRNA approach grape virus control; Micrografting for analysis of effectiveness
    - HP repeated from the promoter story of his talk that they got 20 lines in 8-12 months. Rootstocks were Harmony, Freedom and Salt Creek…don't thinks he discriminated differences in difficulty. Did observe lots of chimeras: 40-60% ("bad surprises")
    - AD noted to do 2 rounds of selection
    - DT indicated a problem with vitrified tissues
    - DT confirmed that Kanamycin is escape prone, while Hygromycin is better
    - AD indicated a concern with needing more oxygenation
    - HP said a second step generation must be on still?
    - AD mentioned disposable bag fermentors. ?? steel…don't know what this meant
    - DT trying to develop Rita (temperature immersion system) for grape. Have success with alfalfa and rice.
- Experiences and approaches
    - Basic source explants to liquid
    - Need data from different programs on their transformation efficiencies and throughput.
- Throughput from different programs
    -
- Experiences with different varieties or lines
    - DT: goal is 10 transformants per construct; Thompson seedless and Freedom are most reliable. Website current offer is Thompson Seedless ($1095/10 transformants) Trying to transform 101-14 w/o success. Also trying 110R
    - CA: doing St. George successfully but with 110R, no organogenesis
    - HP suggested to try using different initials-leaves-internodes; tiny plants in culture to get very young meristematic tissue—HP will share information on how he does it.
- Group applauded HP's offer to share information with pledge to reciprocate

B. Strains of grapevine Xf: the known variation and how it can be exploited for Pierce's disease control (JC Chen, Christopher Wallis, 9 participants)

1) Known Strain Variation
   a) Strain is usually defined on genotype and phenotype (often together), but some genotypes that infect other crops (e.g. almond strains and others, possibly in one case a multiplex) can possibly infect grape, especially in lab settings.
   b) Based on small sample sizes, there is a significant correlation between genotypic and phenotypic distance.
   c) Recombination can occur between grape strains and with multiplex strains, adding diversity to grape strains.
   d) Population diversity of grape strains can be great even in one vineyard (where it has been shown there is low population structure). Viruses, recombination, and through a high incidence of primary inoculation in a single vineyard could result in this observed high diversity.
   e) Still, compared to other *X.f.* strains such as multiplex, grape strains of *X.f.* are more homogenous.
2) How can strain variation be exploited?
   a) Some strains confer increased host resistance against more aggressive *X.f.* grape strains.
   b) Viruses may exist in some *X.f.* isolates which could be used for biocontrol.
   c) However, knowledge of grape strain variation is perhaps most beneficial to understand the success of deploying resistant technologies, such as transformed grape hosts. This is because most studies testing these resistant grapes varieties use only one grape *X.f.* strain, but the diversity of grape *X.f.* strains may already include some strains that can overcome these sources of resistance.
   d) Furthermore, the finding that grape *X.f.* strain diversity is high suggests that aggressive *X.f.* strains may come along and expand their host ranges to infect currently resistant grape species or even other hosts.

Conclusions:

1) Lots of work is still needed to understand *X.f.* grape strain diversity, and what leads to it.
2) Other work examining the frequency of recombination is needed, including work to see if multiple genotypes can exist in the same insect vector or host plant.
3) Biocontrol strains exist, and more might be out there to be discovered.
4) Knowledge of *X.f.* grape stain diversity is needed to properly determine the likelihood that host plant resistance technologies can be overcome.

C. Identities and action mechanisms of Xf proteins contributing to pathogenicity (Dean Gabriel, Zachary Chestnut, 19 participants)

What areas of *Xf* pathogenicity have not been addressed by current or previous research efforts? How could information about these areas be used for PD resistance?

- Major focus areas include polygalacturonase, type I/II effectors.
- What progress has been made into the search for toxins? The current focus areas assume that *Xf* prefers a growth habit that does not kill the plant host.

Is there interest in generating a gene chip, including multiple strains, for profiling expression and changes during infection in compatible and non-compatible hosts?

- RNA from both the bacteria and grapevine should be extracted for joint RNAseq or chip analysis to determine coordinated *Xf* and grape expression changes in such a study.
- Determining what signals or products of *Xf* initiate gene expressing changes in susceptible plant hosts would be helpful.
    - There are multiple sets of gene expression data from the citrus strain (whether they include *Xf* changes, plant changes, or both was unclear). The data exists, but has not been annotated, nor is it available in any database format.

Is laser micro-dissection a feasible tool to investigate gene expression changes in individual cells that have encountered *Xf*, such as xylem parenchyma cells adjacent to infected vessel elements?

- Cells would need to be collected from extensively infected plants in order to find enough candidate parenchyma cells to perform gene expression analysis.
- This approach would enable comparisons of cells that have had direct contact with *Xf* and those further upstream. It can be studied if PD symptom development is linked to the physical presence of the bacteria or some soluble factor that precedes bacterial movement and what plant gene expression changes are associated with each case.

There are merits in going back to a transposon mutagenesis approach to identify what genes are critical for *Xf* for PD development.

- This study would need a model system or other plant system to evaluate disease phenotypes.
    - *Arabidopsis* might provide a good model for determining gene expression changes in the plant, but symptom development would need to be assessed.
    - Grape seedlings show symptoms within 6-8 weeks
- If a key gene for PD development is required for *Xf* growth *in planta*, it would be missed by this type of screen.
    - It could be possible to search for known non-housekeeping genes.

Has anyone knocked out other type II effectors systematically?

- A targeted mutagenesis approach could be used.

It would be beneficial to screen for what other factors are influenced by or play a role in the growth habit switch of *Xf*. A related question is why *Xf* leads to a lethal disease in *V. vinifera* while it lives as an endophyte in many plant species.

- It is possible that *Xf* is growing and spreading too quickly in *vinifera* vessels and hasn't yet evolved the switch to a predominantly endophyte strategy.

- Comparisons between the genomes of the EB92-1 strain and other pathogenic strains will hopefully highlight these factors.
- Transcriptional profiling of the disease habit of *Xf* has been initiated by S. Lindow's group, but outside researchers need to analyze the data to draw conclusions on their own known and characterized pathogenicity factors. Previously unknown factors have not yet been realized.

Different plant host–strain combinations result in different degrees of pathogenicity. These different outcomes can be combined with *Xf* genome and gene expression comparisons to narrow the list of potential pathogenicity factors.

- Preliminary work has shown that an almond strain of *Xf* grows to very low levels in grape with extremely limited symptom development.
    - The *rpfF* mutant of the same almond strain results in severe symptom development when inoculated in grapevines.
        - Will this *Xf* mutant strain have a limited host range or will it cause PD-like symptoms is a wide variety of plants?
        - Does DSF regulate the same genes in different *Xf* strains? (unknown)
            - Is there a motif (i.e., cis-element) regulating DSF that can be compared across multiple strains?
            - The genome sequences of the different strains need to be reevaluated for known DSF targets.
            - Initial investigations into potential DSF levels in strain EB92-1 were inconclusive.
    - Analyzing several strains and their host ranges may not be necessary due to the potential for each strain to cause disease under different regulatory conditions.
        - This would imply that genotype differences between strains would be less important than differences in gene expression.
        - One common factor leading to symptom development in any host-strain combination seems to be the ability of *Xf* to move systemically throughout the xylem.
    - *hxfA* and *hxfB* mutants are both hypervirulent in grape, but *hxfB* is not hypervirulent in almond.
        - Other studied mutant strains perform the same in their respective host-strain combinations; therefore, the differences between HxfA and HxfB sequences in different strains could prove insightful.

Current transgenic rootstock approaches have bypassed any study into what naturally protective products could be translocated from traditional rootstocks.

- Comparing the xylem sap contents of rootstocks and scions could identify factors or signals that would improve translocation efficiency of any transgenic strategies.
- Amino acids or other molecules that influence the growth habit of *Xf* in resistant varieties and non-compatible hosts could be incorporated into rootstocks with the current transgenic strategies.
    - Pyramiding current and future strategies into one, or few, resistant line(s) should provide better long-term resistance.
    - Combining strategies should incorporate differing modes of action – preventative measures (e.g., restricting *Xf* movement within inoculated tissues) vs. toxic approaches.

Current transgenic, rootstock-based strategies could be placed under inducible promoters to allow *Xf* to sufficiently infect inoculated grapevines and the response studied, once the transgenes have been induced.

- This approach would be more beneficial to study the effects of strategies which are toxic to the bacteria as opposed to those which deter or inhibit its movement.
- An alternative approach would be to graft those transgenic rootstocks currently in-hand to scion tissues infected with *Xf*. The changes in *Xf* movement, growth habit, and further symptom development could be analyzed.

D. The potential of viruses and RNA interference in the management of sharpshooters (Bryce Falk, Bryce Falk, 5 participants)

RNAi

Is RNAi technology of potential use for controlling GWSS and other sharpshooters? Would it help to control transmission of Xf to plants? If it can be effective against the sharpshooters, then it would not be so different than would be using pesticides, at least in concept, for helping to control Xf transmission to grapes. Insecticides are used to knockdown GWSS/sharpshooter populations and are non-specific. If RNAi could show effects and help to lower GWSS and other sharpshooter populations, it would be useful, and it would be specific.

How to use it? Probably not by making transgenic grape plants, at least for PD control. Other traits are more important and old varieties/vineyards need to be protected. Maybe use attractant plants, these would be non-grape attractive host plants that would attract and kill or give other negative effects on GWSS helping prevent their movement into grapes. Would it be possible to graft transgenic shoots, or use transgenic rootstocks to deliver RNAi effectors to non-transgenic plant parts? This is unknown, we have not yet demonstrated that transgenic plants themselves can be useful for RNAi on GWSS, this must be done first. We also still are missing fundamental information as to if we can transport effectors through the xylem from a transgenic source.

Could a xylem endophytic microbe be useful for delivering dsRNAs? This is not known. We know that fungi and bacteria can be engineered to produce specific dsRNAs, we don't know if they can be exported outside of the cell.

Sharpshooters primarily feed in the xylem, but do ingest from other tissues during probing etc. Do you need to produce and transport dsRNAs exclusively in the xylem? This is not known and is important and related to how much dsRNA is needed to induce specific effects in sharpshooters. If they need to ingest a lot, then probably xylem delivery is critical.

Viruses

We currently do not know enough about sharpshooter viruses to know their potential. Are sharpshooter viruses pathogens? We do not know the answer to this at present, we do know there are a lot (more than 5, less than 10) viruses so far detected at least in GWSS, good studies on viruses of sharpshooters may be of potential use.

Do the viruses need to be pathogens to be of potential use? Probably not. We may be able to engineer viruses to be more pathogenic (perhaps be expressing insecticidal peptides) and use them to help in sharpshooter control.

How would the viruses be delivered? Can infected sharpshooters be used to spread viruses among the population? These are good questions and relates to the first point above. We don't know enough about the viruses to even know how they spread.

On a related note, can plant viruses be used as vehicles to deliver anti Xf peptides to the xylem? This is not known, and at this time there are not any known viruses that are designed to be used as tools for grapes. But if so, it might be possible to deliver peptides to the xylem using something like Dandekar's xylem specific peptide leader.

E. Novel chemical and other methods for sharpshooter management (Mark Sisterson, Matt Daugherty, 7 participants)

Summary: The group recognized that currently we are reliant on imidacloprid treatments for controlling GWSS. Methods of accentuating the efficacy of biocontrol, landscape management (trap crop/barrier), and plant resistance were all discussed – with no clear recommendations. All group members did agree, however, that there is a great need to **provide to stakeholders a risk assessment and control recommendations for different regions and habitats (vineyards, urban areas, nurseries).**

Treatment thresholds depend on growing region and grower
- -is zero tolerance reasonable?

Alternatives to imidacloprid?
- -imidacloprid is surprisingly effective
- -little to no evidence of insecticide resistance thus far
- -minimal secondary pest outbreaks?
- -do we need to treat every year to achieve adequate control?

Biocontrol
- -needs to be implemented with respect to the chemical control program
- -effective implementation needs to consider alternative resources for natural enemies
- -ants as generalist GWSS predators?

Biocontrol may be a valuable alternative tool for backyard/urban areas
- -probably not going to be very effective
- -impractical to do all the necessary releases

Trap crop approach
- -a trial is ongoing in TX with 25 plant types planted as barrier/trap crops

-for trap crop approach, ideally you want plants that attract them but are poor hosts
-no good candidate plants so far
-GWSS attraction/repellency affected by coloration
-trial in Napa with barrier tree plantings did not result in significant BGSS reductions in vineyards
-perhaps more effective when combined with treatment of barrier plants or use of transgenics in the barrier?

Plant resistance to Xf
-transgenics are a ways off from being widely implemented in management programs
-planting relatively resistant conventional varieties in high risk areas as stop-gap measure?
-resistance not the same (epidemiologically) as tolerance
-growers need more information about what varieties are resistant/tolerant/susceptible
-growers may resist this approach due to consumer demand for certain wines/grapes

F..European Grapevine Moth and Other Invasive Pests and Diseases Threatening California Winegrapes (Beth Stone-Smith, Gevin Kenney, 12 participants)

1 For the European Grapevine Moth, the goal is still eradication.

2 There is concern that other vectors of Xylella could be introduced to California; some of which may be more cold tolerant, and possibly cause Xylella to become a problem farther north in California.

3 Various pests and diseases were discussed; such as the Asian Citrus Psyllid, Red Palm Weevil, Coffee Borer, Brown Marmorated Stink Bugs, Light Brown Apple Moth, Citrus Variegated Chlorosis, as well as others.

4 The conflict with environmentalists over the Light Brown Apple Moth has probably changed how pest management will be conducted in California. It remains to be seen whether the change will be more positive or negative.

5 We discussed improving methods for detecting and identifying new pests. Bugguide.net and whatsthatbug.com were mentioned as good sources of information.

6 There was a strong consensus, probably unanimous, that early detection was very important.

7 We discussed using digital photography for storing data (such as photographing sticky cards rather than storing them), and transmitting data. The Mycro USB Scope IF Series from EmCal Scientific, Inc. was mentioned as a potentially useful and affordable (~ $100) piece of computer hardware for digital macro photography.

8 We discussed the importance of deciding how to best prioritize pests. Since resources are limited and there can be consequences (which can range from uncertain to unknowable) of both acting and not acting; setting priorities can be a very influential and consequential step in the pest management process. How priorities are set can be influenced by such things as industry/commodity groups, concerns of trading partners, crops affected, as well as other political and economic concerns.

9 Industry involvement is critical for pest management in many ways that are not always obvious. For instance, if an affected industry does not seem to be concerned enough about a pest problem to actively attempt to deal with it, then government agencies tend to view the problem as less worthy of action and funding than if the affected industry was clearly taking an active role in dealing with the pest problem.

10 There was a strong consensus, probably unanimous, that we should support and strengthen our entry port protection.